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ADEQUATE Advanced Diagnostics for Enhanced QUality of Antibiotic prescription in respiratory Tract infections in Emergency rooms

Clinical protocol WP4b - Paediatric

VALUE-Dx

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Randomised controlled trial of **Rapid Syndromic Diagnostic Testing** (RSDT) for enhancing the quality of antibiotic prescribing for community acquired acute respiratory tract infection (CA-ARTI) in Emergency Rooms in Europe.

Protocol ID	WP4b - paediatric
Short title	**A**dvanced **D**iagnostics for **E**nhanced **QU**ality of **A**ntibiotic prescription in respiratory **T**ract infections in **E**mergency rooms - **ADEQUATE**
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PROTOCOL SIGNATURE SHEET

Study Title: **A**dvanced **D**iagnostics for **E**nhanced **QU**ality of **A**ntibiotic prescription in respiratory **T**ract infections in **E**mergency rooms – **ADEQUATE**

Version Clinical Protocol: V5.0, 18-Oct-2023

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1. LIST OF ABBREVIATIONS

AE	Adverse event
ARTI	Acute Respiratory Tract Infections
CA-ARTI	Community acquired acute respiratory tract infections
CRP	C Reactive Protein
COVID-19	Coronavirus Infectious Disease 2019
DALY	disability adjusted life years
eCRF	electronic Case Report Form
Dx	Diagnostics
EQ5D	EuroQoL (Europe Quality of Life) 5 dimensions
ER	Emergency Room
ETA	Endotracheal aspirate
GCP	Good Clinical Practice
GPDR	General Data Protection Regulation
ICD	International Classification of Diseases
ICF	Informed Consent Form
MDR	Multidrug Resistant
LAR	Legally Accepted Representative
LRTI	Lower Respiratory Tract Infections
NPS	Nasopharyngeal swabs
PCT	Procalcitonin
POCT	Point of Care Testing
PPAS	Point Prevalence Audit Study
RP2.1. plus	Respiratory Panel version 2.1 plus (BioFire®FilmArray®)
RSDT	Rapid syndromic diagnostic testing
SAE	Serious adverse event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOP	Standard Operating Procedures
SCC	Site selection committee
SSP	Site selection plan
WP	Work Package

2. SUMMARY

Background and Rationale: Community-acquired acute respiratory tract infections (CA-ARTI) are among the most frequent infectious diseases worldwide. At the same time, uncomplicated acute respiratory infections (ARIs) are the most frequent cause of inappropriate antibiotic use. Antibiotic resistance rates are related to antibiotic use in any setting, but opportunities to implement a more judicious antibiotic prescribing are probably most apparent in primary care and emergency departments. Optimal clinical management of CA-ARTI is hampered because of diagnostic delays and suboptimal test sensitivity, leading to incorrect or missing etiologic diagnosis, and over prescription of antibiotics. Highly sensitive molecular assays have increased the detection of respiratory pathogens, but the **impact in clinical decision-making** needs further evaluation. Accurate and rapid identification of infected patients allows for more rational and effective infection control practices and public health responses which will limit morbidity and mortality, economic damage, and can allow low risk/non-infected and recovered people to return to the workforce.

Objective: To assess the impact of rapid diagnostic testing of patients with Acute Respiratory Tract Infection (ARTI) at the emergency department, on (1) hospital admission rates and (2) antibiotic prescriptions (days of treatment). Geographical and seasonal variation will be assessed on a real time basis including pathogens of public health interest. The impact will be stratified within age groups and risk factors in order to determine the long-term clinical, public health and economic determinants for the integration of diagnostics in a global and sustainable perspective.

Study design: Prospective, multi-centre, individually randomised, controlled trial.

Study population: Children of any age consulting in selected participating sites with CA-ARTI with initial uncertainty about management regarding hospitalisation and/or antibiotic therapy.

Study Intervention: The diagnostic intervention is rapid syndromic testing with BioFire FilmArray Respiratory Panel 2.1 plus (RP2.1plus): Nasopharyngeal swab

Main study parameters/endpoints:

- Days alive out of hospital (superiority endpoint), within 14 days
- Days on Therapy (DOT) with antibiotics (superiority endpoint), within 14 days

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participation in the study involves collection of data that can be obtained from medical charts and follow up questionnaires and interviews. Participants randomised to the intervention will receive a nasopharyngeal swab at the ER. This will cause a temporary uncomfortable feeling. Based on the results of diagnostic testing (BioFire FilmArray) antibiotics may be withheld when deemed unnecessary, or a different antibiotic class may be selected when certain bacterial pathogens are detected. The risks and benefits of management decisions, complemented with adequate training, are subject to the current investigation.

3. General considerations Work Package 4 VALUE-Dx overview

The purpose of VALUE-Dx consortium (www.value-dx.eu) is to facilitate and accelerate the rigorous assessment and implementation of (new) diagnostic technologies into healthcare settings, by establishing the infrastructure, methods, processes and approaches needed to understand, evaluate, assess, and demonstrate the multi-faceted value of diagnostics and overcome the associated barriers to their widespread adoption and use. VALUE-Dx focuses its research on **community acquired acute respiratory tract infections (CA-ARTI)**.

The objectives of VALUE-Dx are:

- To design a health-economic framework to assess and demonstrate the value of diagnostics both for individual patients and for public health impact by reducing antibiotic use and subsequent antibiotic resistance among patients;
- To establish a sustainable European Standardised Care Network adequately trained and resourced to conduct clinical trials evaluating the value of diagnostics;
- To design and implement clinical studies to demonstrate the value of diagnostics in the optimal management of CA-ARTIs;
- To explore, define and attempt to resolve the psychological, ethical and social barriers which prevent the more widespread adoption of diagnostics delivering healthcare to the population.

The clinical studies, to be implemented in WP4, aim to gather evidence on:

i) doses or days of antibiotics prescribed,
ii) proportion of patients not receiving antibiotics;

WP4 studies will be performed separately in primary care and long-term facilities (WP4a)- POCT with Abbott and BD and Emergency Rooms (WP4b)- Rapid syndromic diagnostic testing with bioMérieux.

The clinical trial will align – where possible with the other WPs of the project.

- WP1 aims to develop evidence-based clinical algorithms that integrate point of care tests. Initial analysis has provided results on accuracy of the selected groups of items: signs and symptoms, biomarkers, imaging and rapid diagnostic tests, for prediction of influenza and bacterial pneumonia, respectively.
- WP2 will explore analytical performance of some tests in the trials and will collect pathogen and host biomarker data.
- WP3 will provide data management.
- Within WP4a, during the winter season from January to March 2020, a web-based point prevalence audit survey (PPAS) on presentation and management of CA-ARTI in primary care and long-term facilities was performed. The aim is to retrospectively characterise

patients who seek healthcare for CA-ARTI, quantify antibiotic prescriptions and to benchmark patterns of diagnostic in different European countries.

- WP5 is setting-up data collection for health economic modelling, that includes a disease model, diagnostic models and antibiotic resistance predictions. The variables to be included to assess direct and indirect medical costs and quality of life need to be collected in the clinical trials of WP4.
- WP4b, The ADEQUATE study is an individual randomised study that aims to assess the impact of rapid syndromic diagnostic testing (Biofire® bioMérieux) in patients with Acute Respiratory Tract Infection (ARTI) at the emergency department, on (1) hospital admission rates and (2) antimicrobial prescriptions (days of treatment).
- The WP4b clinical protocol has been split into an adult (WP4b – adult) and paediatric population (WP4b – paediatric), to address the differences in standard of care in the two populations, but with common sample size calculation and analysis of the results. The current protocol describes the trial of WP4b for the paediatric population.

4. BACKGROUND AND RATIONALE

4.1. Background

Community-acquired acute respiratory infections (CA-ARTI), including upper and lower respiratory tract infections, are among the most frequent infectious diseases worldwide. Lower respiratory tract infections (LRTI) are among the most lethal communicable diseases and the fourth cause of death globally, responsible for an estimated 3 million deaths in 2016 [1]. LRTI disproportionately affects children younger than 5 years and is the second cause of disability adjusted life years (DALYs). A study published in the Lancet in 2018 estimated the global, regional, and national morbidity, mortality, and aetiologies of LRTI in 195 countries, between the years 1990–2016. In 2016, LRTI caused 652,572 deaths (95% uncertainty interval 586,475–720,612) in children younger than 5 years, 1,080,958 deaths (943,749–1,170,638) in adults older than 70 years, and 2,377,697 deaths (2,145,584–2,512,809) in people of all ages, worldwide *S. pneumoniae* was the leading cause of LRTI morbidity and mortality globally, contributing to more deaths than all other aetiologies combined in 2016 (1,189,937 deaths (690,445–1,770,660)[2], particularly as community acquired pneumonia. Within the European region, geographical variations are present. Over the years, the epidemiology has changed due to changing populations, with increased disease burden in elderly (>70 years) in many regions, varying prevalence of smoking and varying patterns of vaccine usage [3, 4].

On the other hand, uncomplicated ARTI is the most frequent cause of inappropriate antibiotic use [5, 6], and there is a need of more judicious antibiotic prescription to prevent exposure to drug-related adverse events, selection of antibiotic resistance and emergence of opportunistic pathogens that substitute the indigenous microbiota. At the same time, the clinical role of bacteria whose normal ecological niche is the airways is an unresolved issue because of contamination with oropharyngeal flora Antibiotic resistance rates are related to antibiotic use in any setting, but opportunities to decrease unnecessary treatments are probably most apparent in primary care and emergency departments. Not only the ecological but also, the economic cost of antimicrobial resistance per antibiotic consumed is considerable [7-9]. Management is heterogeneous in diverse geographical areas due to non-uniform guidelines, both for diagnostic and antimicrobial stewardship [10].

One of the major challenges in clinical decision making is the absence of microbiological evidence of the aetiological agent in CA-ARTI at the time the antibiotics must be initiated so rapid diagnostic testing may have an impact. The required sample is not always available, and with conventional testing there may be low sensitivity (40-60% cases without aetiological diagnosis) and an important diagnostic delay before results are available. An accurate and reliable distinction between bacterial and viral causes of CA-ARTI would provide an important opportunity for better antimicrobial stewardship. However, due to substantial overlap in clinical disease presentation and laboratory parameters it is currently impossible to reliably distinguish viral from bacterial aetiology based on available tools.

It has been proposed that implementation of Point-of-Care tests (POCT) with biomarkers or microbiological tests to differentiate viral from bacterial infections may reduce inappropriate

antibiotic prescriptions [11]. There is encouraging evidence from randomised trials for biomarkers-guided antimicrobial management. In a multicenter study in adults in primary care the combination of selected clinical symptoms with the addition of C-reactive protein (CRP) measurement improved diagnostic information, but measurement of procalcitonin (PCT) did not add clinically relevant information [12]. In a Cochrane review of controlled trials of biomarkers in patients with CA-ARTI, PCT appeared to be more informative than CRP and other inflammatory markers, as it was associated with an earlier increase upon infection, a better negative predictive value, and was influenced by immunosuppressive medication [13].

The development of highly sensitive molecular assays has increased the detection of respiratory pathogens in patients with CA-ARTI, and increased our understanding of the role of viruses in CA-ARTI [14]. However, diagnostic methods that detect a virus do not always rule-out bacterial infection. Additional diagnostic yield has been demonstrated by using molecular tests but evidence is limited regarding the impact on antibiotic use or costs [15-17].

In this context, a new molecular rapid syndromic testing platform (BioFire; Salt Lake City, UT, USA) might improve clinical decision making in patients with CA-ARTI. The BioFire FilmArray Pneumonia Panel plus (PP) is a multiplexed nucleic acid amplification test that identifies 34 bacterial and viral targets, including antimicrobial resistance genes from sputum or bronchoalveolar lavage (BAL) specimens. The BioFire FilmArray Respiratory Panel 2.1 plus (RP2.1plus) can simultaneously detect 24 viruses and atypical pathogens from nasopharyngeal swabs. Both panels allow syndromic testing and results can be provided in less than one hour with high sensitivity and specificity [18, 19]. Several single-center studies have reported promising results and ongoing clinical trials are summarised in section 9.2. Additional data is needed to prospectively assess the impact of rapid syndromic testing in daily clinical decision making as well as to determine its costs and effects.

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [20] has had an unprecedented and dramatic impact on the health care system and on the world economy. One of the most effective tools in the management of this global pandemic is the ability to rapidly and accurately test patients with signs and symptoms of ARTI or with risk factors for exposure. Some countries have used aggressive and widespread testing paired with contact tracing to manage the crisis and have been apparently the most successful at reducing mortality rates, the strain on the health care system and the spread of the virus. Expanding testing locations could prevent patients from spreading infections due to lack of availability of testing outside hospitals and by reducing the time between sample collection and test result.

4.2. Rationale.

Optimal clinical management of CA-ARTI is hampered because of diagnostic delays and suboptimal test sensitivity, leading to incorrect or missing aetiological diagnosis, and over prescription of antibiotics. Diagnostic and antimicrobial stewardship is important to get the optimal patient management.

There is a need to assess the impact of rapid syndromic diagnostic testing in patients with CA-ARTI presenting to Emergency Rooms on clinical decision making related to:

- Hospitalisation yes or no;
- Start antibiotics yes or no;

At the same time, it must be determined whether the decisions guided by the rapid syndromic diagnostic testing results do not compromise patient safety.

5. OBJECTIVES

5.1. Co-primary objective:

To assess the impact of rapid syndromic testing in patients presenting with CA-ARTI in the ER on:

1. Days in hospital within 14 days after study enrolment
2. Days with antibiotic therapy within 14 days after study enrolment

5.2. Secondary objectives:

1. To assess the impact of rapid syndromic testing on occurrence of adverse outcome within 30 days after study enrolment.
2. To assess the impact of rapid syndromic testing on healthcare utilisation.
3. To assess the impact of rapid syndromic testing on quality of life.
4. To quantify the additional diagnostic yield and sensitivity of rapid syndromic testing because of targets not included in standard of care testing.
5. To quantify the impact of rapid syndromic testing on antimicrobial de-escalation and the choice of antibiotics and prescription of antivirals.
6. To quantify the impact of rapid syndromic testing on detection of antibiotic resistance.
7. To assess the impact of rapid syndromic diagnostic testing on patient bed management and-or isolation measures.

5.3. Exploratory objectives

- To biobank a subset of well characterised clinical samples to encompass epidemiological descriptions of public health interest
- Collect comprehensive data on clinical status and laboratory results for development and validation of clinical algorithms.
- To describe the current routine diagnostic and antimicrobial stewardship policy in Europe and identify good practices
- To estimate the impact of rapid syndromic testing on primary and secondary endpoints for subcategories of hospitals with similar routine diagnostic and antimicrobial stewardship programs.

6. Study parameters/endpoints

6.1. Main study parameter/endpoint

Co-primary study endpoints:

1. Days alive out of hospital (superiority endpoint), within 14 days after study enrolment
2. Days on Therapy (DOT) with antibiotics (superiority endpoint), within 14 days after study enrolment

6.2. Secondary endpoints

Adverse outcome (non-inferiority safety endpoint)

- Safety endpoint:

    - For initially hospitalised patients: i) any readmission, ii) ICU admission => 24 hours after hospitalisation, or iii) death, within 30 days after study enrolment
    - For initially non-admitted patients: any admission or death within 30 days after study enrolment.
- Direct costs and indirect costs within 30 days after enrolment.
- Change in quality of life as determined by EQ-5D-5L (or suitable alternative for age), days away from usual childcare routine or school and healthcare utilisation on day 1, 14, and 30 after enrolment.
- Microbiological results obtained as standard of care and with the diagnostic intervention
- Empirical antibiotics, antibiotic type switches, de-escalation based on antimicrobial agent categories [21]. Prescription of antivirals during the main study.
- Detection of antimicrobial resistance (carriage or infection) related to the diagnostic intervention results compared to standard of care and impact on antimicrobial stewardship guidelines and prevention of hospital acquired infections.
- Impact on decisions regarding isolation measures related to test result.

7. STUDY DESIGN and DURATION

7.1. Study design and justification

This is a prospective, multi-centre, **individually randomised controlled open-label trial.** Approximately 520 subjects will be randomised in the trial in up to 10 investigational sites in the European region. Subjects will be followed at day 14 and day 30 after randomisation.

Enrolment will be competitive across sites.

Justification of the design. The aim is to deliver a study outcome that is **valid** (absence of bias) **precise** (sufficiently powered to achieve clinically relevant absence and presence of difference) and **generalisable** (recognizable population). The study needs to be **feasible** in terms of costs (determined by number of study sites and number of tests to be performed), with an easy patient enrollment and an achievable burden on laboratories. Therefore, although an **individually randomised trial** - in contrast to cluster randomisation - requires informed consent from all recruited patients fulfilling inclusion criteria prior to performing the test. This approach has several advantages: 1. Internal validity of the trial is guaranteed by the randomisation, whereas in cluster-randomised trials, patient selection criteria can be difficult to apply in a consistent manner, inducing a risk of selection bias; 2. Required sample size is lower compared to a cluster-randomised trial; 3. Recognisable population can be realised, although generalisability will always be lower compared to enrolling all-comers; 4. Feasibility: compared to a cluster-randomised trial, a lower sample size is needed, reducing costs for study execution; fewer hospitals, fewer patients, and a lesser burden on labs because tests are only performed in 50% of enrolled patients instead of 100% of all-comers. Moreover, although requirement of informed consent to be obtained at the ER could be considered as a drawback of this design, it in fact may help in enrolling the pursued patient population for this specific

trial. We aim for patients in which the result of the rapid syndromic testing can guide clinical decision making. This implies that there is also time for obtaining informed consent.

In choosing the primary endpoints the following considerations were made:

- The composite endpoint is designed to capture the relevant outcomes and will be combined for analysis
- In the paediatric population the non-inferiority co-primary endpoint is unlikely to be clinically relevant
- Because of the non-blinded nature of the study, outcomes should be defined objectively, so e.g., we cannot use cause-specific re-admission.
- Reassessment when other microbiological results are available. Discrepant results should be handled by the treating physician based on best practice.
- Different time windows have been chosen because i) impact of rapid syndromic testing on hospitalisation / antibiotics is immediate. ii) Shorter follow-up increases power for difference of 1 day. iii) Adverse outcomes may reflect late sequelae (reflected in the key safety secondary endpoint).

Modifications were made following the decision to terminate the recruitment of adult patients (Value Dx WP4b ADEQUATE Protocol, adult version 3.0 18-Feb-2022) on 3rd May 2022. Details are provided in sections 5, 6 and 13 of the current protocol.

7.2. Study duration

The study will encompass at least 3 influenza seasons (autumn/winter months) in Europe.

Justification. i) most CA-ARTI occur in autumn winter months. ii) Timeframes allow for training, initiation and patient follow up. iii) To achieve an adequate sample size iv) Fluctuations in microbiological epidemiology of CA-ARTI (seasonal outbreaks *Mycoplasma*, *Bordetella*, variability on virus lineages, pneumococcal serotypes, etc.) are better represented with more than one season.

The End of Trial is defined as the date of receipt of the last data point from the last subject that is required for primary, secondary and/or exploratory analysis.

8. STUDY POPULATION

8.1. Study population

Children of any age presenting at the Emergency Room in selected participating sites with CA-ARTI, after informed consent by parents or legal guardians and participant's assent where age-appropriate.

8.2. Inclusion criteria

1. **Children** of any age presenting to the **Emergency Room** with an **acute illness** (present for **14 days or less**) with
   Temperature ≥38.0°C measured at presentation or parental report of fever within the previous 72 hours
   AND at least two of the below:
   - Cough
   - Abnormal sounds on chest auscultation (crackles, reduced breath sounds, bronchial breathing, wheezing)
   - Clinical signs of dyspnea (chest indrawing, nasal flaring, grunting)
   - Signs of respiratory dysfunction: tachypnoea for age or decreased oxygen saturation (<92% in room air)
   - Signs of reduced general state: poor feeding, vomiting or lethargy/drowsiness

2. **At time of screening:**
   - Patient has undergone first assessment by managing clinical team (doctor or nurse, incl. triage)
   - Hospitalisation is not yet determined, i.e., neither by clinical presentation definitely requiring hospitalisation (e.g., per local guideline) nor by fixed decision of managing clinical team; admission to a short-stay unit or surveillance unit is not considered a hospitalisation for this trial
   - Antibiotic treatment or hospitalisation is being considered
   - The rapid syndromic diagnostic test result can be awaited up to 4 hours before the decision to discharge the patient or to initiate antibiotic treatment is made

8.3. Exclusion criteria

1. Development of ARTI more than 48 hours after hospital admission (**hospital acquired**);
2. Patients with a severe underlying medical condition dictating management decisions including hospitalisation and/or antibiotic treatment (e.g., **cystic fibrosis, immunosuppression);**
3. Hospitalisation for at least 24 hours within the last 14 days (healthcare-associated);
4. Confirmed pregnancy and/or breastfeeding;
5. Any clinically significant abnormality identified at the time of screening that in the judgment of the Investigator would preclude safe completion of the study or constrain endpoints assessment such as major systemic diseases or patients with short life expectancy;
6. Inability to obtain informed consent;

7. Alternative noninfectious diagnosis that explains clinical symptoms.

8.4. Recommended study site selection criteria

- Does not currently use equivalent rapid testing routinely in patients with CA-ARTI at the ER (rapid defined as time from sample collection to result interpretation by the physician within 4 hours).
- At least 25% of CA-ARTI patients seen at ER are not hospitalised.
- At least 50 paediatric patients with CA-ARTI are seen per month at ER during influenza season
- Microbiology lab is capable of performing molecular testing.
- Highly motivated and GCP-trained local Principal Investigator. Clinical research nurse.
- Chosen timelines for intervention will be considered based on number of consultations and site characteristics. The possibility to obtain real data about costs will be explored.
- **Geographical balance** We will prioritise EU Member States and H2020 Associated Countries with **high and medium antibiotic use** and with a range of **country level income** and **antibiotic stewardship programs**. (if present, the antibiotic stewardship program will be documented, including the list of participant roles). Variations in **microbiological etiologies and vaccination policies** will be taken into consideration.

9. DIAGNOSTIC INTERVENTION

9.1. Description of the test and Intended use

A rapid syndromic diagnostic test - the results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

- **BioFire FilmArray Respiratory Panel 2.1 plus** (RP2.1plus): Nasopharyngeal swab

The **FilmArray Respiratory Panel 2.1 *plus* (RP2.1 *plus*)** is a multiplexed nucleic acid test intended for use with FilmArray® 2.1 or FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The test is FDA approved.
Virus: Adenovirus, Coronavirus (229 E, HKU1, NL63, OC43, SARS-CoV-2), human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Parainfluenza Virus (1, 2, 3, 4), Respiratory Syncytial Virus. **Bacteria:** *Bordetella parapertussis* (IS*1001*), *Bordetella pertussis* (*ptxP*), *Chlamydia pneumoniae, Mycoplasma pneumoniae*

9.2. Summary of findings from clinical studies

The diagnostic test had a high sensitivity (overall 97%) and specificity (overall 99%) in US FDA product registration trials (available from https://www.biofiredx.com/support/documents/ under Instructions for Use and Manuals) and in independent research [23]. In a performance

study that compared the BioFire RP2 Panel to the BioFire RP Panel or PCR and sequencing, the overall percent agreement between the BioFire RP2 Panel and the comparator testing was 99.2% [24].

Biofire respiratory panels:

Several studies have been conducted to examine the clinical impact of the BioFire RP Panel. Findings from such studies included an increase in diagnostic yield, decrease time to diagnosis, lower probabilities of hospital admission, reduced time in isolation, reduced number of supplementary tests such as chest radiographs, reduced hospital length of stay, decreased duration of antimicrobials use, and increase in appropriate antiviral use [25, 26].

As a rapid syndromic diagnostic testing it expedites turnaround time for results, leading to higher rates of early discharge and early discontinuation of antibiotics [27]. In a large retrospective study in paediatric patients with acute viral respiratory tract infections its use was associated with less exposure to antibiotics and chest X-rays and more timely administration of antivirals [28].

9.3. Summary of known and potential risks and benefits

Based on the BioFire FilmArray, antibiotics may be withheld when deemed unnecessary, or a different antibiotic class may be selected when certain bacterial pathogens are detected. These management decisions are made by the patient's treating physician. The risks and benefits of these management decisions are subject to the current investigation.

A risk assessment and monitoring plan will be implemented but based on known intended use of the test (see below). Special effort will be invested on adequate training and monitoring of the sites (both laboratory and clinicians) for an optimal use of the test, and to support in the clinical and therapeutic decisions based on test results.

Safety and warning precautions of the product are summarised in the Instructions for Use as well as the Summary of Results and Limitations, with focus on the following:

- Asymptomatic carriage of viruses does occur, or the virus could be a co-pathogen together with a bacterial pathogen, or a recent viral infection could have predisposed to a secondary bacterial pneumonia.
- Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test and pathogens below the limit of detection
- There is a risk of false positive results due to non-specific amplification and/or cross-reactivity with organisms found in the respiratory tract and they are summarised in the Instructions for use. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.

Expected **benefits of the study** are, together with the main objective of reducing hospitalisation rates and antimicrobial use without adverse outcome, the opportunity for improved training, harmonised improvement of existing guidelines (local and European), opportunity to grow the different existing networks, opportunity to improve surveillance

because of underreporting or not available diagnostic tests. Assessing the diagnostic test in children is very important given the high rate of antimicrobial prescription when presenting with respiratory symptoms and the scientific research may directly benefit this target population. The potential benefit is much bigger than the risk of withholding a treatment when it should be given, which is a remote situation (<1%).

10. METHODS

10.1. Screening and enrolment

Patients in the emergency department and/or short-stay unit will be screened for eligibility by local coordinators, clinicians, nurses in collaboration with members of the research team.

Study staff will seek informed consent from all patients meeting the eligibility criteria at the time of screening. The health status of patients might rapidly deteriorate between screening and randomisation. Therefore, all eligibility criteria need to be re-evaluated and confirmed prior to the decision to randomise the patient.

Screening failures are defined as patients, who were found eligible per screening but have either not given informed consent, or have deteriorated between screening and randomisation, and therefore no longer fulfil eligibility criteria. Screening failures are recorded anonymously on a screening log detailing the reason for screening failure. They are not randomised. Of note, no diagnostic procedures will be performed for the purpose of checking eligibility criteria specifically, i.e., any procedures indicated for the standard of care patient management will be performed but none will be added in order to check eligibility criteria.

10.2. Randomisation and blinding

Due to the nature of the intervention, blinding is not possible. After all eligibility criteria have been verified and informed consent has been obtained, randomisation will be performed using the built-in randomisation module of the eCRF system. Allocation will be concealed until the moment of randomisation. To this end, block randomisation will be used with variable blocks of size 2, 4 and 6. Randomisation will be stratified by centre. After the decision to randomise the subject is made, subjects will not be excluded from the trial. If the allocated intervention is not applied for any reason, this will be recorded and follow-up for the participant will be completed.

10.3. Data collection

A secured electronic case record form (eCRF) will be specifically designed for this study.

- **Main study** (Follow up until 30 days) – Standard of care clinical and microbiological data will be collected but no study specific biological samples will be obtained with the exception of the diagnostic test in the respective allocation group. The **clinical data set** will summarise the illness episode and outcome, microbiological testing and antimicrobial use including the total hospitalisation, or in case of discharge, the time window defined on the primary endpoints. Follow-up information including data for

health economic analysis will be collected on day 14 (visit window: day 12 – 16) and on day 30 (visit window: day 28 – 32) after randomisation. Parents/legal guardians/participants (where age-appropriate) will be asked to consent to being contacted by study staff for the follow-up visits to minimise loss to follow-up. In case of failure to successfully contact parents/legal guardians/participant (where age-appropriate) at the end of trial participation, the participant's general practitioner/family doctor will be contacted to complete information on the primary endpoints.

- **Microbiology study** Clinical data will be collected as defined above. At participating study sites, additional biological samples as specified in section 10.7 will be collected, provided separate informed consent is given. Day 30 follow-up will include a face-to-face visit for participants in the microbiology study.

10.4. Clinical data set

Inclusion. Check for inclusion and exclusion criteria.
Randomisation. Informed Consent Form. Baseline registration and investigations.
Signs and symptoms
at ER and management plan.
Participant background.
Vaccination
Co-morbidities
and chronic medication
Standard of care haematology and biochemistry
EQ-5D-5Y questionnaire
Management: Clinical decision after randomisation and initial results
Investigations: respiratory, urine, faeces, blood, SARS-CoV-2, radiology (only when standard of care)
Day 14: symptoms, EQ-5D-5Y questionnaire
Day 30: symptoms, EQ-5D-5Y questionnaire
Outcomes and safety:
Antibiotic treatment
Antiviral and antifungal agents
Hospital course
Device deficiency
(Severe) Adverse Event
Deviation
End of study

Day 14 and 30 follow-up
Days away from normal daily routines, healthcare utilization, antibiotic prescriptions, any hospital admission, ICU admission or death.

Table 1. Visit Schedule

Procedures	Screening	Enrollment	Hospitalisation Period[e]	Discharge[e]	Online questionnaire, phone call or face-to-face visit	
	Pre- study procedures Day 1	Day 1	> Day 1		Day 14	Day 30
Informed consent	X					
Eligibility criteria	X					
Demographics[a]	X					
Baseline assessment[b]	X					
Vaccination status	X					
Comorbidities and chronic medications	X					
Hospital course[c]		X	X[e]	X[e]		
Microbiological testing[d]		X				X
Concomitant therapy review	X	X	X[e]	X[e]	X	X
Symptoms review and hospitalizations	X	X	X[e]	X[e]	X	X
Quality of life questionnaire EQ5D		X		X[e]	X	X

a Age in months or years date of birth, sex, weight, date of admission, partial postal code

b Symptoms, signs at clinical examination, duration of complaints, patient attending ER spontaneously or referred by general practitioner

c ICU admission, hospital length of stay, ICU length of stay, non-invasive ventilation, vasopressor use, extracorporeal support
d Assessments only performed if patient is hospitalized
e Only applicable when informed consent is obtained for the additional study

10.5. Baseline and follow-up data for health-economic analysis

Follow up Assessments: Timepoints: Day 1, Day 14 and Day 30

Individual patient data will be clustered in several main categories, and will reflect patient status prior to consultation (including quality of life assessment), diagnostics (including microbiological diagnostic tests and thoracic imaging), medicines (antibiotics and any other prescribed medication at the ER/admission including several parameters, antibiotic prescription in the previous 14 days) and patient follow-up (quality of life, adherence to treatment, duration of complaints –diary- and productivity costs including productivity loss and disability and designed differently for adults and children).

Quality of life- (see eCRF completion guidelines on guidance**)** The EQ-5D (https://euroqol.org) contains 5-dimensions ("5D") related to everyday living: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Patients rate the problems they have with each of these dimensions on a 5-level scale ("5-L") from 1 (no problems) to 5 (extreme problems). The second part of the EQ-5D-5L asks patients to grade their current global health status from 0 (worst health you can imagine) to 100 (best health you can image). The questionnaire form, takes ± 5-10 min to administer. It needs to be completed by patient or person who has known the patient for > 5 years. (Order of preference: caregiver > spouse > children 18+ > sibling > other acquaintance). For patient questionnaires, patients will receive a link per email to complete the questionnaires online, which will directly be incorporated into the eCRF and be regarded as source data once digitally completed by the patients. If this is not possible or if patients are seen face to face, questionnaires will be completed per paper (source data) and entered in the eCRF by study staff at the site. In this case paper versions will be stored as source data. Quality of life data for the paediatric trial population will be captured using age-appropriate tools, including proxy questionnaires for completion by parents or guardians for younger children.

10.6. Microbiological testing

Results from standard of care testing. Microbiological tests according to standard of care from site.

Known previous colonization with multidrug resistant microorganism.

Known previous SARS-CoV-2 infection if required hospitalisation and data are available

- Type of samples collected: blood, ETA, BAL, pleural fluid, urine
- Type of tests: cultures, quality markers for sputum (<10 epithelial cells and >25 leukocytes per field magnification x 100), antigen detection, antibody detection, molecular testing
- Test results: list of microorganisms. Selected susceptibility patterns

Susceptibility testing will be grouped based on antimicrobial resistant phenotypes definitions: methicillin resistant *Staphylococcus aureus* –MRSA-, carbapenem resistant Enterobacteriaceae, Extended Spectrum Beta Lactamases Enterobacteriaceae –ESBL-, carbapenem-nonsusceptible *Pseudomonas aeruginosa*, multidrug resistant *P. aeruginosa*.

(Based on https://www.cdc.gov/nhsn/PDFs/pscManual/11pscAURcurrent.pdf)
The data collected will allow also reporting pathogens of public health interest.
Within VALUE-Dx WP3 there is current work on inter-operability with lab devices, with the objective to achieve direct connectivity with these systems to have antimicrobial resistance information from lab results easily available.

Study samples. For diagnostic intervention: nasopharyngeal swab. Specific procedures for collection and processing will be provided. Data to be recorded:

- Test result (including data on genomic copies provided by the test)
- Time at which Test Result was Generated
- Time at which Test Result was received by care team and how it is communicated
- Time at which antibiotics were prescribed
- Results from standard of care diagnostic tests (e.g., cultures) with comparable targets and assessment of discrepant results

10.7. Microbiology study and biobanking

In a subset of study sites and participants (up to 150 participants), additional oropharyngeal samples will be obtained from participants. One sample will be obtained on the day of randomisation and one sample on day 30 (visit window: day 28 – 32) after randomisation. Specific procedures for collection and processing will be provided. The aim of the microbiology study is to use suitable methods, including metagenomic sequencing, to characterise changes in microbiological colonisation and antimicrobial resistance patterns dependent on treatment with antibiotics. Inclusion in the microbiology study will require separate informed consent and participation in the main study will not depend on consent for the microbiology study.

Biological samples obtained for the study (including leftovers from the specimens obtained for the intervention and for the microbiology study) can be stored at all sites and shipped to the University of Antwerp for inclusion in a biobank, subject to the condition that separate informed consent for biobanking is given. Participation in the study will not depend on consent for biobanking.

11. Training

11.1. Good Clinical practice training.

The Principal Investigator and all site personnel involved in the study need to provide a valid ICH-GCP certificate to timely identify and enrol eligible patients, collect informed consent forms, collect source data, enter data into clinical database. See the ADEQUATE monitoring plan for more details. Guidelines will preferably follow ISO20916:2019 because of the specific differences in device related events and deficiencies reporting.

11.2. Medical Device training, being part of the Site Initiation Visit.

Training of the hospital staff involved in the study will be implemented, with an emphasis on sample collection, sample processing, correctly identifying causative organisms and performance of susceptibility testing in coordination with bioMérieux. Where required,

guidance will be provided to improve procedures and align them with standardised uniform manners following the principles of Good Clinical Laboratory Practice (GCLP). Quality Control of the device will be performed as described in the ADEQUATE monitoring plan.

11.3. Data management training

All sites will be trained to use the data management system (Research Online) as part of the site initiation visit. To assure high quality the Data Management Department of the Julius Centre (JC) of UMCU works according to a Quality Management System. All work is carried out in accordance with UMCU written Standard Operating Procedures (SOP) and Work instructions.

12. SAFETY REPORTING

12.1. Adverse events

Definitions

Adverse Event (AE)

For this study, an adverse event is defined as any untoward medical occurrence, inappropriate patient management decision, unintended disease or injury, or untoward clinical signs in subjects, users, or other persons, with any connection to study related activities, whether or not related to the IVD medical device.

Adverse device effect (ADE)

An adverse event related to the use of the BioFire®.

Serious Adverse Event (SAE)

All SAEs, regardless of relationship to the study device or procedure will be documented in the source. It is the Investigator's responsibility to determine the "seriousness" of an AE using the protocol defined terms, listed below. A SAE is an AE that results in one or more of the following for this study:

- Resulted in death: An AE that resulted in the patient's death.
- Life-threatening illness or injury: The patient was at imminent risk of dying at the time of the adverse event.
- Permanent impairment: An AE that resulted in permanent impairment of a body function, including chronic diseases or permanent damage to a body structure.
- Required in-patient or prolonged hospitalisation.
- Resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or body function.
- Led to fetal distress, fetal death or congenital abnormality or birth defects, including physical or mental impairment.

Notes:

1. Hospitalisation on Day-1 should not be reported as an AE or SAE if this is a direct consequence of the initial referral to the hospital.
2. SAEs resulting in death should be reported using the primary cause of death as the event term. The only exception is "Sudden Death" when the cause is unknown.
3. Planned hospitalisation for a pre-existing condition is not considered a SAE.

Serious adverse device effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Categories of adverse events

ADVERSE EVENTS	**Non-device-related**	**Device-related**
		Applies to: - Inaccurate test result leads to indirect harm to the subject - Device causes direct harm to user or another person
Non-serious	Adverse event	Adverse device effect
Serious	Serious adverse event	Serious adverse device effect

SEVERITY OF ADVERSE EVENTS

It is the Investigator's responsibility to assess the severity of an AE. A change in severity may constitute a new reportable AE.

Also, the following guideline should be used to determine the severity of each adverse event:

- MILD: Awareness of signs or symptoms, but does not interfere with the patient's usual activity, or is a transient event that resolves without treatment and with no sequelae.
- MODERATE: A sign or symptom, which interferes with the patient's usual activity.
- SEVERE: Incapacity with inability to do work or usual activities.

RELATIONSHIP OF ADVERSE EVENTS

It is the Investigator's responsibility to assess the relationship between all AEs to the study device and procedure. The following guidelines should be used in determining the relationship of an AE to a device, procedure, or other causality:

Not related	Relationship to the procedures or device can be excluded when: • The event is not a known side effect of the product category the device belongs to or of similar device and procedures;

	• The event has no temporal relationship with the use of the device or the procedures; • The event involves a body-site or an organ not expected to be affected by the device or the procedure or the disease under investigation; • The event can be attributed to another cause (e.g., an underlying or concurrent illness/clinical condition, an effect of another device, drug, treatment, or other risk factors); • Harms to the subject are not clearly due to use error; or • To establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedure and the event.
Unlikely	The relationship with the use of the device seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.
Possible*	The relationship with the use of the device is weak but cannot be ruled out completely. Alternative causes are also possible (e.g., an underlying or concurrent illness/condition and/or an effect of another device, drug, or treatment). Cases where relatedness cannot be assessed, or no information has been obtained should also be classified as possible.
Probable*	The relationship with the use of the device seems relevant and/or the event cannot reasonably be explained by another cause, but additional information may be obtained.

Causal Relationship*	The event is associated with the device or with procedures beyond reasonable doubt when:
	• The event is a known side effect of the product category the device belongs to or of similar device and procedure
	• The event has a temporal relationship with the device uses/application or procedures
	• The event involves a body-site or organ that
	  ○ The device or procedures are applied to
	  ○ The device or procedures have an effect on
	• The event follows a known response pattern to the medical device (if the response pattern is previously known)
	• Other possible causes (e.g., an underlying or concurrent illness/clinical condition and/or an effect of another device, drug, or treatment) have been adequately ruled out
	• Harm to the subject is due to error in use
	• To establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedure and the event.

*Denotes “related” to the study procedure or device and should be reported (AE) as part of the study.

12.2. Reporting Adverse Events

The principal investigator shall:

a) record all AEs and device deficiencies, regardless of relationship to the study device or study procedure in the source, together with an assessment as to whether the device or study procedure were a cause of the event,

b) report to the Sponsor, without unjustified delay (max. 24 hours after becoming aware of the event), all SAEs and SADEs, regardless of relationship to the study device or procedure, from the time of signing the informed consent through study completion (day-30 follow-up visit, including window, for the main study and the microbiology study) and device deficiencies that could have led to a SADE; this information shall be promptly followed by detailed written reports, as specified in the Safety Management Plan,

c) report to the Sponsor, without unjustified delay (max. 7 calendar days after becoming aware of the event), all AEs, that deemed to have a possible, probable, or causal relationship to the study procedure, or device, from the time of signing the informed consent through study completion (day-30 follow-up visit, including window, for the main study and the microbiology study); this information shall be promptly followed by detailed written reports, as specified in the Safety Management Plan,

d) ensure reporting of SAEs or SADEs to the ethics committee, or if applicable a competent authority, per the timelines of the national regulations, from the time of signing the informed consent through study completion (day-30 follow-up visit, including window, for the main study and the microbiology study) and device deficiencies that could have led to a SADE, when required by the national regulations or Safety Management Plan or by the ethics committee,

e) ensure annually reporting to the ethics committee, or per the timelines of the national regulations, AEs that deemed to have a possible, probable, or causal relationship to the study procedure or device, from the time of signing the informed consent through study completion (day-30 follow-up visit, including window, for the main study and the microbiology study), when required by the national regulations or Safety Management Plan and

f) ensure to report to regulatory authorities, SADEs and device deficiencies that could have led to serious adverse device effect, as required by national regulations, and supply the Sponsor, upon Sponsor's request, with any additional information related to the safety reporting of a particular event. Sponsor remains responsible for adequate reporting to regulatory authorities.

The study site will report applicable safety events to the Sponsor by entering the event into the AE form in the eCRF, which will trigger an automated email to the Sponsor and manufacturer. Refer to the ADEQUATE Safety Management Plan for more extensive guidance on reporting.

12.3. Device Deficiency

Device Deficiency Definition

A device deficiency is defined as inadequacy of the BioFire® and/or kits with respect to its identity, quality, durability, reliability, usability, safety or performance.

Device deficiencies include, but are not limited to, malfunctions, use errors, and inadequacy in the information supplied by the manufacturer including labelling.

A device deficiency may or may not be associated with an AE/SAE.

Reporting Device Deficiency

All device deficiencies related to devices in the procedure shall be documented throughout the study. The study site must report device deficiencies related to the BioFire and/or kits, within 24 hours after becoming aware of the event through the Device Deficiency form in the eCRF.

Manufacturer representatives will organise collection of the device for evaluation, as needed.

12.4. DEVIATIONS FROM THE CLINICAL STUDY PROTOCOL

A protocol deviation is any non-compliance with the study protocol, Good Clinical Practice. A deviation can be identified from a number of sources. Potential sources include, but are not limited to, a member of the Investigator's staff, a Sponsor representative during monitoring visits, or a member of the data management or statistical groups when entering or analyzing data. Regardless of the source, it is crucial to document the deviation in the protocol deviation eCRF. The Investigator will report protocol deviations to the IRB/EC as required by the IRB/EC procedures.

Any deviation from the protocol or procedures should be recorded in the source documents. Standard of care assessments not completed at a site should not be considered protocol deviations.

Steps to be taken to assure the accuracy and reliability of data include the selection of qualified investigators and appropriate sites, the review of protocol procedures with the Investigator and associated personnel prior to the study, and periodic monitoring visits by the Sponsor. The Sponsor will review eCRFs for accuracy and completeness during on-site and/or remote monitoring visits; any discrepancies will be resolved with the Investigator or designees, as appropriate.

12.5. Data Safety monitoring board (DSMB)

A Data Safety Monitoring Board has been established with the aim to safeguard the interests of trial participants, assess the safety of the interventions during the trial, and monitor the overall conduct of the trial. A specific charter document describes the roles and

responsibilities of the independent DSMB for the ADEQUATE trial, including the timing of meetings, methods of providing information to and from the DSMB, frequency and format of meetings, statistical issues and relationships with other committees.

13. STATISTICAL CONSIDERATIONS

13.1. Sample size calculation – joint paediatric and adult trial (protocol version 2.0, 23-Nov-2020 (paediatric)/protocol version 3.0, 18-Feb-2022 (adult))

Co-primary endpoints used in the study:

1. Days on antibiotic treatment within 14 days (superiority endpoint)
2. Days alive out of the hospital within 14 days (superiority endpoint)
3. Clinical failure within 30 days (non-inferiority safety endpoint)

Required sample sizes are presented for endpoint 2 and 3. Endpoint 1 is non-limiting due to having an expected smaller standard deviation compared to endpoint 2 and having the same clinical relevant effect size.

The intervention is considered successful if superiority is demonstrated for either or both endpoints 1 and 2 AND non-inferiority is demonstrated for endpoint 3. We use a hierarchical nested design: superiority primary endpoints are tested first. Only if superiority to at least one of the two superiority endpoints is confirmed, the non-inferiority safety endpoint is taken into consideration. To maintain an overall alpha < 0.05, the two superiority endpoints will be tested using a two-sided alpha of 0.025, while the non-inferiority endpoint will be tested using a one-sided alpha of 0.05. The minimal power for the superiority endpoints is set to 0.9 and for the non-inferiority endpoint to 0.95. This is to maintain overall power of the trial, given the hierarchical testing. For 'Clinical failure' we considered different non-inferiority margins. For 'Days on antibiotic treatment' and 'Days alive out of the hospital' we defined 1 day as a clinically relevant effect size.

The distribution of endpoint 2 is yet unknown for the ARI population. We supported our sample size calculation using data of patients presenting with CAP to Dutch ERs and hospitalised to a non-ICU ward (ClinicalTrials.gov: NCT02604628). The standard deviation (SD) of 'Days alive out of the hospital within 14 days' in this population was 4 days. The theoretical maximum SD with a 14-day follow-up period is 7 (which would be the case if 50% had zero days and 50% had 14 days alive out of the hospital). However, we expect that in the target population the variability for this endpoint will be less than in the CAP population and in children less than in adults. We varied the SD to assess the impact on the sample size. We used a standard correction factor of 1.15 for non-normality of the data. (Table 2) Hence for the superiority endpoints a sample size of 257 per arm for children would be adequate.

Table 2: Sample sizes for Days alive out of the hospital using different assumptions

SD	Relevant effect size	Alpha	Beta	Correction	Sample size per arm

3	1	0.025	0.1	1.15	257
4	1	0.025	0.1	1.15	457
5	1	0.025	0.1	1.15	714
6	1	0.025	0.1	1.15	1,028
7	1	0.025	0.1	1.15	1,399

For clinical failure, if we assume an incidence of 5% it would be 412, using a non-inferiority margin of 5% (Table 3). A high power for clinical failure is considered important because both the superiority and the non-inferiority hypothesis need to be confirmed in order to declare the intervention a success so a total of 412 participants per study arm would be required to reach 95% power

Table 3: Sample sizes for clinical failure using different assumptions.

Incidence *	Non-inferiority margin	Alpha**	Beta	Sample size per arm
0.05	0.05	0.05	0.05	412

* same for intervention and control arm; ** one-sided alpha

To account for potential drop-outs we originally set an aim of a **total of 900 randomised children** in the study.

13.2. Review of sample size calculation for the current version of the protocol

The sample size calculation was redone after the decision to terminate the recruitment of adult patients (Value Dx WP4b ADEQUATE Protocol, adult version 3.0 18-Feb-2022) on 3rd May 2022. We first considered the three co-primary endpoints listed in 13.1.

Co-primary endpoint EP3: Clinical failure within 30 days (non-inferiority safety endpoint)
The non-inferiority safety endpoint, defined as any inpatient admission or death within 30 days after study enrolment for initially non-admitted patients (expected to be >90% of paediatric participants) and any readmission, secondary ICU admission or death within 30 days after study enrolment for initially admitted patients, is unlikely to be relevant or appropriate for the paediatric population considered alone.

Rationale: Mortality in the study population is extremely low, and secondary admission rates among children initially managed in the community as well as readmission and secondary ICU admission rates among primarily admitted children are in fact likely to be in the range of 2-2.5%. Secondary admissions are highly unlikely to be related to the initial illness episode due to the relative conservative approach in current paediatric emergency medicine practice erring on the side of primary admission if there is any doubt about appropriate safety netting being possible for the family. Based on this, EP3 will be considered a key secondary endpoint for the statistical analysis plan and will no longer be used to inform the target sample size.

Co-primary endpoints EP1 and EP2: Days on antibiotic treatment and Days alive out of hospital, both within 14 days (superiority endpoints)
The original assumptions for EP1 and EP2 remain unchanged. In particular, a reduction of one day in antibiotic treatment or increase of one day in days alive out of hospital appear to be relevant for a clinically relevant reduction in antibiotic prescribing and a reduction in hospital costs, respectively. In children, the co-primary superiority endpoints are likely to be dominated by EP1, as ambulatory exposure to antibiotics is likely to be common in the absence of hospital admission, whereas many admitted children would be expected to be treated with antibiotics as well. The superiority comparison on EP1 will be considered the primary outcome comparison for the trial.

The sample size estimation was revisited for co-primary endpoint EP1. From a recent publication on variations in antibiotic prescribing in febrile children presenting to European EDs, the standard deviation for days on antibiotic treatment was estimated as 3.7 days. Based on this, recruitment of 170 children per arm (total of 340 children) will be sufficient to detect a difference of one day in EP1 (power 80%, alpha 0.05; table 4).

Table 4: Sample sizes for Days on antibiotic treatment (paediatric) using different assumptions.

SD	Delta	Alpha	Beta	Correction	Sample size per arm
2.5	1	0.025	0.2	1	99
3.0	1	0.025	0.2	1	142
3.5	1	0.025	0.2	1	193
3.7	1	0.025	0.2	1	215
4.0	1	0.025	0.2	1	252
4.5	1	0.025	0.2	1	318
5.0	1	0.025	0.2	1	393

To account for uncertainty about the variability in EP1 (and EP2) in the paediatric study population, we propose to adopt a highly conservative approach aiming to recruit 252 children per arm (total of 504 children), resulting in adequate power to detect a difference in one day in EP1 or EP2. To adjust for loss to follow-up of up to 10%, we will aim for a **total of 554 randomised children**.

13.3. Analysis populations

A statistical analysis plan will be prepared after protocol approval and once site selection process has been initiated.

The main analyses will be performed for the total population

Analyses will be stratified by:

- Country/geographical location
- age groups (i.e., <5y/5-17)
- clinical syndrome: clinical pneumonia/LRTI, influenza-like illness, laryngitis/laryngotracheitis (croup), dominant obstructive airway disease/bronchiolitis
- risk factors: patients with and without known risk factors for severe disease; the latter include chronic pulmonary, cardiovascular or metabolic disease or immunocompromised patients.
- severity at presentation: patients with and without primary hospital admission.

- Vaccination status: Completed primary vaccinations according to local schedule y/n, pneumococcal vaccine receipt according to local schedule y/n, receipt of influenza vaccine for current season y/n, SARS-CoV-2 vaccine receipt y/n.

The primary analysis will follow the intention-to-treat principle in which groups are compared based on the allocated regimen. In the per protocol analysis, we will exclude participants not receiving the test according to the randomised regimen (e.g., randomised to rapid syndromic testing but not receiving the test).

13.4. Primary study parameter(s)

Descriptive statistics will be produced and tabular summaries will be presented, stratified according to the allocated group (rapid syndromic testing vs. control). Categorical data will be summarised by the number and percentage of subjects in each category. Appropriate summary statistics will be used for continuous variables depending on the distributional assumptions. These include measures of central tendency (mean or median) and dispersion (standard deviation or inter-quartile range).

'Days on antibiotic treatment' and 'Days alive out of the hospital' will be analysed using a linear regression analysis, including as covariates stratification by age groups and predefined risk factors. Upfront we may expect that the assumption of normally distributed residuals is violated, in which case we will determine the confidence interval by bootstrapping.

13.5. Secondary study parameter(s)

Clinical failure will be analysed using Cox proportional hazards regression adjusted for age groups and predefined risk factors. Risk differences and 90% CI will be inferred by comparing the cumulative incidence of failure at day 30 from the Cox model and bootstrapping for the confidence interval. A 90% CI will be used to be compatible with a one-sided alpha of 0.05.

Economically relevant parameters will be gathered within the trial context from individual participants but also the following data from the participant sites/countries will be extracted when possible:

- Patient numbers relative to population and hospital catchment area
- Estimations on the number of emergency department visits associated with acute respiratory infections based on a list of ICD codes.
- For the long-term economic model, the probability of susceptibility to received antibiotics and the resulting illness duration, additional outpatient visits and a second antibiotic course will be estimated based on surveillance data on every site. When needed, assumptions regarding the incidence and hospitalisation rates will be derived from literature and expert opinion

Types of costs: Direct medical costs, direct and indirect non-medical costs.
Hospital costs will be determined using bottom up calculation based on representative sites that have the information available on electronic databases that allow **unit cost prices** assignment to the variables recorded. These descriptive data will include health care utilization for the entire hospitalisation, for ICU stay (unit cost price per hospitalisation day,

unit cost price for ICU hospitalisation day). Unit cost price for recorded diagnostic tests and thoracic imaging (referred to the procedure of testing as coded/reimbursed by official nomenclature).
When not available, resource use will be measured as volume of hospital admission days, tests performed, etc.
Analysis of the antibiotic domain based on antibiotic type switches and de-escalation. For the health economic analysis, a decision-tree **deterministic** approach with specification of the base case and alternative scenarios, together with threshold analyses to determine efficient ranges for the values of some parameters. Analysis of the antibiotic domain based on antibiotic type switches and de-escalation.

13.6. Mid-term data analysis

A data snapshot will be taken after the first season, in terms of number of recruited cases per category, impact of seasonal outbreaks or emerging pathogens. Sample size assumptions may also need to be validated.

14. ETHICAL CONSIDERATIONS

14.1. Regulation statement

The study will be performed in accordance with all applicable laws and regulations including the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP) the ethical principles that have their origins in the Declaration of Helsinki (current official version: Fortaleza, 2013; https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-researchinvolving-human-subjects/), the updated version of the General Data Protection Regulation (EU) 2016/679 (GDPR), ISO 20916 and other applicable privacy laws.

14.2. Recruitment and consent

The investigator or authorised delegate must obtain written informed consent before any clinical study related procedure/activity takes place. Parents or guardians and participants will be approached for the study by the local principal investigator or appropriately trained delegate, in co-ordination with the attending clinical team. Written versions of the Participant Information Sheet and Informed Consent will detail the exact nature of the study; the implications and constraints of the protocol; and any risks involved in taking part. It will be stated clearly in the information letter that participation in this study is completely voluntary and that withdrawal is possible at any time and without any consequences. Consent will be sought from one or both parents in accordance with local regulations. Where the participant has the capacity to contribute to the decision about participation, they will be involved in the informed consent process in an age- and capacity-appropriate way. This will include obtaining assent to participation where appropriate. The standard consent form will request consent from subjects for sample storage of specific samples to collaborating institutions for investigations that cannot be performed locally. Staff will explain the details of the study to

the participant or parent/guardian/consultee and allow them time to discuss and ask questions. The consenting party will be asked to sign and date an informed consent form. Obtaining a subject's consent to participate in medical research may be complicated by COVID-19 measures. In the case of the patient being in isolation and unable to sign, he/she will give his/her oral consent, the researcher will sign directly, and the patient will sign as soon as possible after completion of the isolation period. Deviations in consent procedures might occur per participating country, following national law and locally accepted procedures with regards to challenging COVID-measures.

Participant Information Sheets will be available in the common local language. Written Informed Consent will be confirmed by the dated signatures of the participant and by the person who presented and obtained the informed consent. A copy of the signed Informed Consent will be given to the participants. The original signed form will be retained at the study site. Following the code of conduct for pediatric patients, in all medical research involving child subjects, the burden associated with participation should be minimised; where non-therapeutic research is concerned, the law stipulates that it must be negligible.

14.3. Withdrawal of individual subjects

Patients are free to withdraw consent at any time without providing a reason. Patients who wish to withdraw consent for the study will have anonymised data collected up to the point of that withdrawal of consent included in the analyses. The patient will not contribute further data to the study. Data up to the time of withdrawal will be included in the analyses unless the patient appeals to the 'right to be forgotten' according to the national GDPR regulations. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Enrolled patients meeting one or more of the exclusion criteria *prior* to randomisation, will be withdrawn by the investigator. Patients withdrawn prior to randomisation will be replaced (i.e., they will not count towards the sample size). Patients withdrawn after randomisation will not be replaced.

The participants cannot be enrolled at the same time into any interventional clinical study unless it is a COVID-19 study that does not interfere with this study. Subjects may be co-enrolled in another observational study if the local study coordinators have been informed and have given their approval, to ensure the other study would not interfere with the results of this study or compromise patient welfare.

15. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

15.1. Steering Committee

The missions of the steering committee are to define the objectives of the research, to propose protocol modifications during the research, to organise the research, to determine the methodology, to coordinate information and to monitor the conduct of research. The steering

committee will decide during ongoing research what to do in unexpected situation. The steering committee will meet regularly until the end of inclusions.

15.2. Handling and storage of data and documents

Data will be recorded in a secured electronic case record form (eCRF) specifically designed for this study and validated for authenticity, accuracy, reliability and consistent intended performance. An eCRF should be completed for each participant.

All information obtained during the study (except the ICF data) will be entered digitally in conformity with the applicable laws and regulations. All data (except the ICF data) will be coded using a unique study number. Data will be handled in accordance with local privacy regulations and the European General Data Protection Regulation (GDPR, 2016/679, effective as of May 25th, 2018). Penta as Sponsor of the study will have access to all data collected from the start of the study

At the end of the study all data will be transferred to Penta Foundation and will be stored for 25 years.

15.3. Storage of samples. Biobanking

Biological samples will be obtained from a subset of participants as detailed in section 10.7. Detailed instructions for sample storage will be provided separately. Samples will be stored at local facilities in a pseudonymised way until shipment to the central trial laboratory.

15.4. Monitoring and Quality Assurance

Remote/centralised and/or on-site study monitoring will be carried out by the Sponsor. A monitoring plan will be scheduled and will define the monitoring frequency and procedures. Monitoring will start before recruitment begins, throughout the trial (data monitoring including but not limited to recruitment rates, consent procedures, access/storage of patient identifiers, sample handling for performing the diagnostic intervention test, data entry, data queries, unusual data patterns), between recruiting season and at the end of the trial (source document verification, collect trial supplies, close-out meetings/visits). We will use a risk adapted approach within the protocol design to enable safety reporting requirements to reflect the amount of safety data available and the level of risk for this non-interventional study. We refer to the monitoring plan for details.

This study does not involve any experimental treatment, but the diagnostic intervention may interfere with the standard of care and it involves data collection. The need for additional insurance for study patients may differ per country local regulations.

15.5. Public disclosure and publication policy

All information disclosed or provided by the Sponsor (or any company/institution acting on their behalf) or produced during the study, including, but not limited to, the protocol, the eCRF, and the results obtained during the course of the study, is confidential prior to the publication of results and in accordance to consortium agreement and open access regulations.

The detailed procedures for the review of publications are set out in the clinical trial agreement entered into with the Sponsor in connection with this study. These procedures are in place to ensure coordination of study data publication and adequate review of data for

publication against the validated study database for accuracy. UMCU will adhere to all applicable local laws governing transparency in clinical trials including the trial posting on clinicaltrials.gov and all other applicable registrations.

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17. Appendix 1: Summary of Changes

	Version Number	Version Date
Current Approved Protocol	V2.0	13-Nov 2020
Amended Protocol	V3.0	11-Nov-2022
Amended Protocol	V4.0	26-Jan-2023
Reviewed Amended Protocol	V5.0	18-Oct-2023

Section number, title and page number	Changes	Rationale for change
Cover page	**Addition of "Julia Anna Bielicki" and "Malte Kohns Vasconcelos" to protocol contributors**	**Developed new protocol**
Study overview	Moved study acronym to end of study title	Clarity
Study overview	Amended protocol version/date	For new protocol
Study overview	Change of Coordinating Investigator from *"Cristina Prat Aymerich"* To *"Malte Kohns"*	Change in sponsor
Study overview	Change of Chief Investigator from *"Marc Bonten, UMC Utrecht"* To *"Julia Bielicki, St George's, University of London"*	Change in sponsor
Study overview	Change of sponsor from *"University Medical Center Utrecht (UMC Utrecht)* *Heidelberglaan 100* *3584 CX Utrecht The Netherlands"* To *"Fondazione PENTA ONLUS* *Torre di Ricerca Pediatrica* *Corso Stati Uniti, 4* *35127, Padova* *Italy"*	Change in sponsor
Study overview	Members of Steering Committee	Change of format
Study overview	Added "*Andrew Atkinson, UKBB*"	Additional statistician worked on amendment
Study overview	Added members of DSMB	Update to members
Protocol signature sheet:	Change of sponsor signatory from *"Prof. Dr. K.G.M. Moons"* To	Change in sponsor

	"*Laura Mangiarini*"	
Protocol signature sheet:	Change of text from *"Principal Coordinating Investigator Prof. Dr. M.J.M Bonten Microbiologist, UMC Utrecht"* To *"Principal Investigator Julia Bielicki, Senior Lecturer St George's, University of London"*	Change in chief investigator
Protocol signature sheet:	Removal of Penta Coordinating Investigator	Change in sponsor – no longer relevant
Protocol signature sheet:	Addition of individual pages for signatures	Signature page for each site provided
Protocol signature sheet:	Added additional sites and investigators	Updated sites list
Table of Contents:	Amended "*Value-Dx*" to "*VALUE-Dx*"	For consistency across project
Table of Contents:	Amended page numbers	Due to pages shifting
Table of Contents:	Change of text from: *"Study site selection criteria"* To *"Recommended study site selection criteria"*	More appropriate title of the paragraph that follows
Table of Contents:	Change of text from: *"Laboratory training"* To *"Medical device training, being part of the Site Initiation Visit"*	More appropriate title of the paragraph that follows
2. Summary	Addition of "*acute respiratory infections*"	Clarity
2. Summary	Amended "*antimicrobial*" to "*antibiotic*"	Grammar correction
2. Summary	Removal of "and (3) the non-inferiority in terms of clinical outcome"	No longer a primary endpoint
2. Summary	Change of text from: *"Individually randomised controlled trial. Hierarchical nested analysis design."* To *"Prospective, multi-centre, individually randomised, controlled trial."*	Clearer and more specific definition.
2. Summary	Following text removed: "*Adverse outcome (non-inferiority safety endpoint)* *For initially non-admitted patients: any admission or death within 30 days* *For initially hospitalised patients: i) any readmission, ii) ICU admission >= 24 hours after hospitalisation, or iii) death, all within 30 days*"	This endpoint is no longer a primary endpoint – now a secondary endpoint

2. Summary	Following text removed: *"and who do not produce sputum"*	Not applicable to all of the paediatric population.
3. General considerations:	Amended "*Value-Dx*" to "*VALUE-Dx*"	For consistency across project
3. General considerations:	Removal of "and (3) the non-inferiority in terms of clinical outcome"	No longer a primary endpoint
4.1 Background:	Amended "*effects*" to "*affects*"	Spelling correction
4.1 Background:	Amended "*prescribing*" to "*prescription*"	Grammar correction
4.1 Background:	Amended "*ecologic*" to "*ecological*"	Grammar correction
4.1 Background:	Addition of *"The Biofire"*	Full commercial name
5.1 Co-primary objective	Removal of "*occurrence of adverse outcome within 30 days after study enrolment*"	Not applicable to paediatric study
5.2 Secondary objectives:	Insertion of *"to assess the impact of rapid syndromic testing on occurrence of adverse outcome within 30 days after study enrolment."*	This was a primary endpoint but is now a secondary endpoint
6. Study parameters/ endpoints.	Moved *"Adverse outcome (non-inferiority safety endpoint)* • *Safety endpoint:* • *For initially hospitalised patients: i) any readmission, ii) ICU admission => 24 hours after hospitalisation, or iii) death, within 30 days after study enrolment* • *For initially non-admitted patients: any admission or death within 30 days after study enrolment."* from 6.1 Main study parameter/endpoint to 6.2 Secondary endpoints	In paediatric population it is unlikely this endpoint will be clinically relevant
6.1 Co-primary study endpoints:	Following text added after each of the two co-primary study endpoints: "*after study enrolment"*	Additional information added regarding the study endpoints.
6.2 Secondary endpoints:	Following text added: *"Change in"* (second bullet point) *"during the main study"* (fourth bullet point)	Additional information regarding the secondary endpoints.
7.1 Study design and justification:	Change of text from: *"The study design will be an individually randomised controlled open-label trial"* To *"This is a prospective, multi-centre, individually randomised controlled open-label trial. Approximately 520 subjects will be randomised in the trial in up to 10 investigational sites in the European region. Subjects will be followed at day 14 and day 30 after randomisation.* *Enrolment will be competitive across sites."*	Clearer and more specific definition. Test includes change to sample size and number of investigational sites from previous protocol due to removal of adult sites. Clarification of the enrolment type.
7.1 Study design and justification:	Change of text from: "*lab both in adult and paediatric sites*" To "*laboratories*"	Removed reference to adult sites

7.1 Study design and justification:	Change of text from: *"It needs to be ensured that the non-inferiority endpoint is not positively impacted by direct effects of the intervention such as decisions to initiate or withhold antibiotic treatment or early discharge from the ER or hospital. E.g., if we would include hospitalisation days in the non-inferiority endpoint, we run the risk of 'compensating' the putative adverse effects."* To *"In the paediatric population the non-inferiority co-primary endpoint is unlikely to be clinically relevant"*	Change in primary/ secondary endpoints
7.1 Study design and justification:	Removal of text: *"Hierarchical nested design: Superiority primary endpoints are tested first. Only if superiority to at least one of the two superiority endpoints is confirmed, is the non-inferiority safety endpoint taken into consideration"*	Non-inferiority safety endpoint no longer a primary endpoint
7.1 Study design and justification:	Removal of text: *"or cause-specific death"*	Removal of death as primary objective
7.1 Study design and justification:	Addition of text: "*reflected in the key safety secondary endpoint"*	Composite endpoint summarises the most relevant adverse events including death
7.1 Study design and justification:	Addition of text: "*Modifications were made following the decision to terminate the recruitment of adult patients (Value Dx WP4b ADEQUATE Protocol, adult version 3.0 18-Feb-2022) 3rd May 2022. Details are provided in sections 5, 6 and 13 of the current protocol".*	Closure of adult arm of trial
7.2 Study duration:	Change of text from "*2 influenza seasons*" To "*3 influenza seasons*"	Study has been extended
7.2 Study duration:	Following text added: *"(autumn/winter months)"*	Clarification of the meaning of "influenza seasons".
7.2 Study duration:	Removal of text: *", however timelines might be extended related to the COVID 19 situation"*	In line with changes made to the whole paragraph.
7.2 Study duration:	Addition of text: *"The End of Trial is defined as the date of receipt of the last data point from the last subject that is required for primary, secondary and/or exploratory analysis."*	Clearer definition of the study duration.
8.1 Study population	Change of text from: *"consulting"* To *"presenting at the Emergency Room"*	Clearer description of the study population.

8.2 Inclusion criteria	Change of text from: *"Temperature ≥38.0°C measured at presentation or reported within the previous 24 hours"* To *"Temperature ≥38.0°C measured at presentation or parental report of fever within the previous 72 hours"*	The key function of the fever requirement in the inclusion criteria is to separate children with infection from those with non-infection-related respiratory symptoms that may otherwise present in similar ways (e.g. asthma). As fever is only present during a short period of time especially in younger children who mostly have viral causes of their infection, we extend the possible interval between fever and enrolment.
8.3. Exclusion criteria	Change of text from: *"Less than 14 days since the last episode of respiratory tract infection"* To *"Hospitalised for at least 24 hours within the last 14 days (healthcare-associated)"*	Clarity
8.4 Recommended study site selection criteria (title)	Following text added: *"Recommended"*	More specific definition of the paragraph title.
8.4 Recommended study site selection criteria (first bullet point)	Change of text from: *"Does not currently use equivalent rapid testing routinely in patients with CA-ARTI (rapid defined as time from sample collection to result interpretation by the physician within 4 hours). Specific SARS-CoV-2 molecular testing will be considered individually."* To *"Does not currently use equivalent rapid testing routinely in patients with CA-ARTI at the ER (rapid defined as time from sample collection to result interpretation by the physician within 4 hours)."*	Clarification of the place where the rapid testing routinely is performed. Removal of sentence regarding SARS-CoV-2 molecular testing.
10.1 Screening and enrolment	Following text added: *"and confirmed"*	Clarification of the actions to take before

		enrolling new participants
10.2. Randomisation and blinding	Following text added: *"the subject"*	Clarity
10.2. Randomisation and blinding	Change of text from: *"patients"* To *"subjects"*	Consistency
10.3 Data collection	Following text removed: *"We will collect detailed demographic and clinical data from patient history and examination, patient and caretaker questionnaires and electronic chart reviews. Extension of the required data collection will be adjusted according to stratification and time points."*	Text in the paragraph that follows the sentences removed already explains data collection in detail
10.3 Data collection	Change of text from: *"Main study (Routine clinical and microbiological data collection and follow up until 30 days) – Clinical data will be collected but no biological samples will be obtained for research purposes with exception of the diagnostic test in the respective allocation group, if this sample is not taken as per standard of care."* To ***Main study*** *(Follow up until 30 days) – Standard of care – clinical and microbiological data will be collected but no biological samples will be obtained with the exception of the diagnostic test in the respective allocation group.*	Clarification of the description of the main study
10.4 Clinical data set	Change of text. The whole paragraph has been reviewed. Table 1 Visit Schedule added.	Clearer explanation of Clinical data set
10.5 Baseline and follow-up data for health-economic analysis	Following text added: *"(see eCRF completion guidelines on guidance)"*	Reference to a more detailed document
10.5 Baseline and follow-up data for health-economic analysis	Change of text: *"To"* To *"It needs to"*	Clarity
10.5 Baseline and follow-up data for health-economic analysis	Following text moved to this paragraph: *"For patient questionnaires, patients will receive a link per email to complete the questionnaires online, which will directly be incorporated into the eCRF and be regarded as source data once digitally completed by the patients. If this is not possible or if patients are seen face to face, questionnaires will be completed per paper (source data) and entered in the*	Text moved to a more appropriate section of the protocol

	eCRF by study staff at the site. In this case paper versions will be stored as source data."	
10.6 Microbiological testing.	Change of text from: "*is*" To "*are*"	Grammar correction
10.7 Microbiology study and biobanking	Following text removed: "at 3 study sites"	Harmonisation with the rest of the protocol
11.1 Good Clinical practice training.	Change of text from: *"Site personnel will be trained to obtain a Good Clinical practice (GCP) certification (if not already done) to timely identify and enrol eligible patients,"* To *"The Principal Investigator and all site personnel involved in the study need to provide a valid ICH-GCP certificate to timely identify and enrol eligible patients,"*	Clearer explanation of one of the responsibilities of the Principal Investigator in relation to GCP training
11.1 Good Clinical practice training.	Following text added: *"See the ADEQUATE monitoring plan for more details."*	Reference to a more detailed document
11.1 Good Clinical practice training.	Following text removed: *"Specific training for the follow up questionnaires will be implemented if needed."*	Removal of sentence regarding training for follow up questionnaires
11.2 Medical Device training, being part of the Site Initiation Visit.	Change of text from: *"Laboratory training"* To *"Medical Device training, being part of the Site Initiation Visit."*	More appropriate title of the paragraph that follows
11.2 Medical Device training, being part of the Site Initiation Visit.	Change of text from: *"laboratory"* To *"the hospital"*	Better explanation of who is receiving training
11.2 Medical Device training, being part of the Site Initiation Visit.	Following text removed: *"During site selection, lab external accreditation and participation in External Quality Assessments will be recorded."*	Removal of sentence regarding lab accreditation
11.2 Medical Device training, being part of the Site Initiation Visit.	Following text added: *"Quality Control of the device will be performed as described in the ADEQUATE monitoring plan."*	Reference to a more detailed document
11.3 Data management training.	Change of text from: *"our"*	Clarity

	To *"UMCU"*	
12.1 Adverse events	Change of text from: *"2. Progression of the disease under study should not be reported as an SAE.* *3. "Death" should not be reported as an AE. The cause of death should be reported as an AE. The only exception is "Sudden Death" when the cause is unknown."* To *"2. SAEs resulting in death should be reported using the primary cause of death as the event term. The only exception is "Sudden Death" when the cause is unknown."*	Clearer explanation of what constitutes a Serious Adverse Event (SAE)
12.2 Reporting Adverse Events	Following text removed: *"disease under investigation"*	Paragraph aligned with paragraph 12.1
12.2 Reporting Adverse Events	Following text added: *"Sponsor remains responsible for adequate reporting to regulatory authorities."*	Clarification of one of the responsibilities of the Sponsor in relation to reporting Adverse Events
12.2 Reporting Adverse Events	Change of text from: *"AEs and all SAEs"* to *"safety events"*	Clarity
12.2 Reporting Adverse Events	Following text added: *"Refer to the ADEQUATE Safety Management Plan for more extensive guidance on reporting."*	Reference to a more detailed document
12.2 Reporting Adverse Events	Following text removed: *"Additional information, including the Investigator's assessment, may be added to the eCRF later. Any necessary medical management of the event will be recorded in the patient's medical record/source document.* *If the Sponsor requires supporting documentation or other information, the Sponsor will contact the study site.* *Data related to AEs and SAEs will be collected until event resolution, until the event is considered stable, or until all attempts to determine the resolution of the event are exhausted.* *All AEs and SAEs that are unresolved at study completion (or early termination) will be recorded as ongoing at study end.* *In addition, the following information should be recorded:* • *Onset date* • *Resolution date or date of death* • *Action taken* • *Event status (ongoing at study end or resolved)* • *Relationship of AE to the BioFire device used in the study* • *Relationship of AE to the sampling procedure* • *Indication of seriousness*	Removal of text regarding AEs and SAEs

	• *Was AE anticipated or not (only for serious, device-related AEs)"*	
12.4 Deviations from the clinical study protocol	Change of text from: *"A protocol deviation is any noncompliance with the study protocol, Good Clinical Practice, or protocol-specific requirements. A deviation (any activity conducted outside the parameters established by the study protocol) can be identified from a number of sources."* To *"A protocol deviation is any non-compliance with the study protocol, Good Clinical Practice. A deviation can be identified from a number of sources."*	Removal of text regarding protocol deviation
13.1 Sample size calculation:	*Addition to section title - "joint paediatric and adult trial (protocol version 2.0, 23-Nov-2020 (paediatric)/protocol version 3.0, 18-Feb-2022 (adult))"*	Kept in to show initial sample size calculations, title amended for clarity
13.1 Sample size calculation:	Change of text from *"we will aim for"* To *"we originally set an aim of"*	Change in sample size
13.2 Sample size calculation.	Addition of text *"Review of sample size calculation for protocol version 3.0, 11-Nov-2022: paediatric study* *The sample size calculation was redone after the decision to terminate the recruitment of adult patients (Value Dx WP4b ADEQUATE Protocol, adult version 3.0 18-Feb-2022) on 3rd May 2022. We first considered the three co-primary endpoints listed in 13.1.* *Co-primary endpoint EP3: Clinical failure within 30 days (non-inferiority safety endpoint)* *The non-inferiority safety endpoint, defined as any inpatient admission or death within 30 days after study enrolment for initially non-admitted patients (expected to be >90% of paediatric participants) and any readmission, secondary ICU admission or death within 30 days after study enrolment for initially admitted patients, is unlikely to be relevant or appropriate for the paediatric population considered alone.* *Rationale: Mortality in the study population is extremely low, and secondary admission rates among children initially managed in the community as well as readmission and secondary ICU admission rates among primarily admitted children are in fact likely to be in the range of 2-2.5%. Secondary admissions are highly unlikely to be related to the initial illness episode due to the relative conservative approach in current paediatric emergency medicine practice erring on the side of primary admission if there is any doubt about appropriate safety netting being possible for the family. Based on this, EP3 will be considered a key secondary endpoint for the statistical analysis plan and will no longer be used to inform the target sample size.*	Change in sample size

	Co-primary endpoints EP1 and EP2: Days on antibiotic treatment and Days alive out of hospital, both within 14 days (superiority endpoints) *The original assumptions for EP1 and EP2 remain unchanged. In particular, a reduction of one day in antibiotic treatment or increase of one day in days alive out of hospital appear to be relevant for a clinically relevant reduction in antibiotic prescribing and a reduction in hospital costs, respectively. In children, the co-primary superiority endpoints are likely to be dominated by EP1, as ambulatory exposure to antibiotics is likely to be common in the absence of hospital admission, whereas many admitted children would be expected to be treated with antibiotics as well. The superiority comparison on EP1 will be considered the primary outcome comparison for the trial. The sample size estimation was revisited for co-primary endpoint EP1. From a recent publication on variations in antibiotic prescribing in febrile children presenting to European EDs, the standard deviation for days on antibiotic treatment was estimated as 3.7 days. Based on this, recruitment of 170 children per arm (total of 340 children) will be sufficient to detect a difference of one day in EP1 (power 80%, alpha 0.05; table 4).* *To account for uncertainty about the variability in EP1 (and EP2) in the paediatric study population, we propose to adopt a highly conservative approach aiming to recruit 251 children per arm (total of 502 children), resulting in adequate power to detect a difference in one day in EP1 or EP2.* *To adjust for loss to follow-up of up to 10%, we will aim for a total of 554 randomised children."* Addition of table 4 – "sample sizes using different assumptions"	
13.2 Sample size calculation.	Change of text from *"Review of sample size calculation for protocol version 3.0, 11-Nov-2022: paediatric study"* To *"Review of sample size calculation for the current version of the protocol"*	More appropriate title of the paragraph that follows
13.3 Analysis populations.	Change of text from: *"Analyses will be stratified by:* • *age groups (i.e. <5y/5-17)* • *clinical syndrome: exacerbation of chronic pulmonary disease, influenza-like illness, laryngitis/laryngotracheitis (croup), acute bronchitis/bronchiolitis (depending on age)* • *risk factors. Patient groups at risk for developing severe disease are well known, such as the elderly, patients with chronic pulmonary, cardiovascular or metabolic disease or immunocompromised patients. Clinical predictors of severity are also well known. Still, within risk groups may exist the uncertainty whether to hospitalise and/or to treat and the impact of testing may be assessed.* • *Vaccination status. Influenza. Pneumococcal."*	Addition of variables for stratification

	To *"Analyses will be stratified by:* • *Country/geographical location* • *age groups (i.e., <5y/5-17)* • *clinical syndrome: clinical pneumonia/LRTI, influenza-like illness, laryngitis/laryngotracheitis (croup), dominant obstructive airway disease/bronchiolitis* • *risk factors: patients with and without known risk factors for severe disease; the latter include chronic pulmonary, cardiovascular or metabolic disease or immunocompromised patients.* • *severity at presentation: patients with and without primary hospital admission.* • *Vaccination status: Completed primary vaccinations according to local schedule y/n, pneumococcal vaccine receipt according to local schedule y/n, receipt of influenza vaccine for current season y/n, SARS-CoV-2 vaccine receipt y/n."*	
13.4 Primary study parameter(s)/13.5 Secondary study parameter(s).	Text below moved from primary to secondary study parameters *"Clinical failure will be analysed using Cox proportional hazards regression adjusted for age groups and predefined risk factors. Risk differences and 90% CI will be inferred by comparing the cumulative incidence of failure at day 30 from the Cox model and bootstrapping for the confidence interval. A 90% CI will be used to be compatible with a one-sided alpha of 0.05."*	Change in primary/ secondary endpoint
13.6 Mid-term data analysis	Change of text from: *"A data snapshot will be taken at the end of month six, after the first season,"* To *"A data snapshot will be taken after the first season,"*	Clarification of time when mid-term data analysis will be performed
14.1 Regulation statement	Following text added: *"ISO 20916"*	Additional standards added to the list of regulations to abide to
14.2 Recruitment and consent	Following text added: *The investigator or authorised delegate must obtain written informed consent before any clinical study related procedure/activity takes place."*	Clarification of obligation to receiving written IC before performing any study related procedure
14.2 Recruitment and consent	Following text removed: *"The investigator or delegate will be trained in consent procedures that protect the rights of the patient and adhere to the ethical principles within the Declaration of Helsinki."*	Removal of sentence regarding training in consent procedures
14.2 Recruitment and consent	Following text removed: *"and verbal"*	Sentence aligned with first sentence of this paragraph
14.2 Recruitment and consent	Following text removed: *"Therefore, it is proposed to obtain ICF from LAR or in presence of an impartial witness to confirm ICF is verbally*	Sentence no longer applicable in view of

	given where it is not possible to obtain written consent directly."	the first sentence of this paragraph
14.2 Recruitment and consent	Following text added: *"Deviations in consent procedures might occur per participating country, following national law and locally accepted procedures with regards to challenging COVID-measures."*	Clarification of procedure for receiving consent during the COVID-19 pandemic
14.3 Withdrawal of individual subjects	Following text added: *"unless it is a COVID-19 study that does not interfere with this study."*	Clarification of co-enrolment of participants into another interventional study during the COVID-19 pandemic
15.1 Steering Committee	Change of text from: *"every 6 months"* To *"regularly"*	Clarification of the frequency of the steering committee's meeting
15.2 Handling and storage of data and documents	Following text removed: *"For patient questionnaires, patients will receive a link per email to complete the questionnaires online, which will directly be incorporated into the eCRF and be regarded as source data once digitally completed by the patients. If this is not possible or if patients are seen face to face, questionnaires will be completed per paper (source data) and entered in the eCRF by study staff at the site. In this case paper versions will be stored as source data."*	Text moved to a more appropriate section of the protocol
15.2 Handling and storage of data and documents	Addition of text: *"Penta as Sponsor of the study will have access to all data collected from the start of the study. At the end of the study the Data will be transferred to Penta Foundation and will be stored for 25 years."*	Change in sponsor
Throughout	General administrative corrections throughout: Addition of "*the*" Amendment of "E.g." to "E.g.'" Amendment of "I.e." to "I.e.'"	Grammar corrections
Throughout, Footer	Amendment of protocol version and date	For new protocol
PI and site names	Changed the St George's Hospital PI from 'Dr Louise Hill' to 'Dr Louisa Brock'	Change in PI
PI and site names	Removed Padua and Greek site that are not taking part in the study	Change in site list
Throughout, Footer	Amendment of protocol version to V5.0 and date 18-Oct-2023	For new protocol
Study summary table	Added the NCT number	Information missing

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